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GENETIC ASPECTS OF RESTORING OLYMPIA OYSTERS AND OTHER NATIVE BIVALVES: BALANCING THE NEED FOR ACTION, GOOD INTENTIONS, AND THE RISKS OF MAKING THINGS WORSE

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ABSTRACT As interest and efforts in ecological restoration of native bivalve populations grow, the genetic implications of various restoration strategies are often unclear to resource managers and restoration practitioners, even though genetic considerations are vital to the ultimate success or failure of restoration endeavors. In an effort to fill this void, we present an overview of the underlying genetic concepts, a brief review of documented examples of native mollusc populations impacted by hatchery production, and a summary of the potential genetic impacts of restoration activities ranging from eliminating ongoing negative impacts with minimal genetic effects to intentional genetic manipulation of extant populations. We emphasize throughout the importance of understanding how adaptive, quantitative genetic variation is distributed within and among populations and the limitations of studies that address only selectively neutral molecular genetic variation. We also describe a conceptual framework for making genetically sound management and restoration decisions based on historical and current ecological and genetic considerations. Finally, because fully-informed decisions require a great deal of difficult-to-obtain data, we make suggestions on how to prioritize future research and outline practical measures that can be implemented in the absence of rigorous genetic data to prevent inadvertent negative genetic impacts by well-intended restoration efforts.

KEY WORDS: Quantitative genetics, local adaptation, restoration, genetic rehabilitation, effective population size, oyster, *Ostrea lurida*, Olympia oyster

INTRODUCTION

In many areas of the world, wild oyster and other bivalve populations have been decimated by unsustainable harvesting, habitat destruction, pollution, and disease. Olympia oysters (*Ostrea lurida* Carpenter 1864)† the only oyster species native to the United States west coast, is no exception. This species, historically a major component of estuarine fauna and the basis of important subsistence fisheries, was commercially extirpated in California by the 1860s and throughout the Pacific Northwest by about the year 1900 largely as a result of over harvesting and the effects of siltation caused by mining and logging activities (Baker 1995, Barnett 1963, Breese & Wick 1974, Browning 1972, Jackson 1979, Kirby 2004)

Recent research efforts have clearly demonstrated the importance of some oyster species as foundation species in estuarine ecosystems and generated interest in restoring native oyster populations and the ecological benefits they may provide, including water filtration and essential habitat for a wide variety of invertebrates and fish (Breitburg et al. 2000, Coen & Luckenbach 2000, Lenihan et al. 1999, Lenihan et al. 2001, McCay et al. 2003, Micheli & Peterson 1999, Peterson et al. 2003, Zimmerman et al. 1989). In the Chesapeake Bay, for example, it has been estimated that before the collapse of Eastern oyster populations, the species was capable of filtering the entire volume of that enormous estuary in just a few days (Newell 1988). In Tomales Bay on the west coast, the presence of native oysters is associated with higher species diversity of benthic invertebrates (Kimbro 2004, Kimbro & Grosholz 2006), but other ecosystem services have not been studied. Peterson et al. (2003) estimated that restoring 10 m^2 of oyster reef in the southeastern United States could result in an additional 2.6 kg yr^{-1} of fish and large crustacean production.

At present, Pacific oysters (*Crassostrea gigas*) are cultured in many areas formerly populated by Olympia oysters and are a valuable commercial product. Seed oysters collected from the few naturalized populations or produced in hatcheries can be grown to market size in most west coast estuaries, but environmental conditions are seldom suitable for reproduction and recruitment resulting in "put-and-take" culture operations, typically on a limited scale compared with the former abundance of the native species (Breese & Wick 1974). As a result, farmed Pacific oysters are not capable of providing the same level of ecological services as the native oyster species. A key difference between the two species is how they reproduce and disperse. Olympia oyster females brood their fertilized embryos within their mantle cavities for up to two weeks and release well-developed veliger larvae whereas Pacific oysters release unfertilized gametes that develop entirely in the water column. As a result, with a few notable exceptions, Pacific oysters have not become naturalized in west coast estuaries because even in places where they do spawn, their larvae are flushed into the open ocean.

Hatchery and nursery techniques developed in the 1970s and 1980s for Pacific oyster aquaculture are now being used to supplement extant populations or re-establish Olympia oysters in parts of their historical range (S. Cudd, S. Evans, D. VanderShaff, S. Van der Wetering pers. comm.). At the ecological level, this strategy seems straightforward and attractive

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†The taxonomy of the Olympia oyster has been in dispute since Harry (1985) proposed synonymy of *Ostrea lurida* Carpenter 1864 and *Ostrea conchaphila* Carpenter 1857. Polson et al. (2009) provide molecular evidence that the Olympia oyster refers to the nominal species, *Ostrea lurida* Carpenter 1864. In view of their genetic data, and for consistency, the original taxon, *Ostrea lurida*, is used throughout this volume to refer to the Olympia oyster, which is distributed from approximately Baja California (Mexico) to southeast Alaska.

given that the major goal of native oyster restoration efforts is to rebuild their numbers and thereby re-establish the ecosystem functions they once provided and potentially even a viable fishery. From a genetic perspective, however, supplementation through hatchery culture is more complex, and it is even possible that well-intentioned efforts to augment native oyster populations through transplantation or hatchery production could negatively impact the very populations targeted for restoration by altering their genetic composition in ways that compromise their long-term viability.

This is possible for at least two reasons. First, if the existing levels and patterns of genetic variation are the product of local adaptation to specific environmental conditions, and if these locally-adapted populations harbor sufficient genetic variation to respond to future challenges, hatchery-based population supplementation with inappropriate genetic stocks could reduce fitness, eliminate valuable genetic variation, or even completely replace adapted wild genotypes with nonadapted alien ones. On the other hand, existing patterns of genetic variation within and among populations have conservation value only if they contribute to long-term potential for adaptation and persistence (Crandall et al. 2000, Lande 1999, Lande & Shannon 1996, Pearman 2001). Thus, if extant patterns of genetic variation represent a nonadaptive product of demographic history or geography (especially of detrimental anthropogenic effects) or adaptation to past environmental conditions that are unlikely to return, extant patterns of genetic variation may not have conservation value. In such cases, perpetuating artificially low genetic diversity, inbreeding, and/or nonadaptive patterns of genetic differentiation could actually limit or prevent future adaptation to changing conditions and thus increase the likelihood of local extinction. Of course the key question is which scenario applies. To the extent that this question can be answered at all, only carefully designed and implemented empirical studies can settle the matter. It is important to appreciate, however, that proceeding in ignorance, especially on a large scale, could have lasting consequences, be they for better or for worse.

In this paper, we summarize the relevant genetic concepts; provide an overview of the potential genetic impacts of restoration efforts; and make suggestions to guide future research, restoration, and management. Our goal is to do so in a compact format accessible to resource managers and restoration practitioners, because they are charged with making the critical decisions ''on-the-ground'' that determine the nature, extent, and severity of the genetic impacts of restoration efforts. In the sections that follow, we first review the principles of population-level genetics as a foundation for a discussion of how restoration efforts, especially hatchery-based supplementation, can alter the genetic composition of natural populations. Our treatment of this vast literature is necessarily somewhat cursory and admittedly idiosyncratic because of space limitations. For those interested in a more thorough but very accessible treatment, we recommend Conner and Hartl's excellent primer (Conner & Hartl 2004). We then describe the options available to restoration efforts, ranging from doing nothing to deliberate manipulation of the genetic make-up of populations and outline how they might affect native oyster populations. We follow this with a brief review of documented examples of native mollusc populations having been impacted by hatchery production and a conceptual framework for making genetically sound management and restoration decisions. Unfortunately fully-informed decisions require a daunting amount of difficult-to-acquire empirical data to implement, so we also present some practical measures that can be implemented with or without these data to prevent inadvertent negative genetic impacts.

OVERVIEW OF GENETIC PRINCIPLES

Forms of Genetic Variation

Quantitative Genetic Variation

Before the development of molecular genetic tools, evolutionary biologists and plant and animal breeders developed sophisticated theoretical and statistical approaches to understanding the degree to which differences in the measurable characters of organisms (phenotypes) are determined by inherited factors (genotypes) *versus* environmental influences (Falconer & Mackay 1996, Lynch & Walsh 1998 are excellent texts). In the most general terms this quantitative genetic approach involves evaluating the extent to which the degree of genetic similarity among individual organisms is predictive of their phenotypic similarity, with genetic similarity determined by familial relationships such as brother/sister, parent/offspring, first cousins, and so on. That is, quantitative genetics aims to analyze quantitatively the familiar qualitative observation that relatives resemble each other more than nonrelatives, and in doing so to provide rigorous analyses of the genetic composition of populations, the selective forces that generated these configurations, and predictions about the way in which they will respond to natural and artificial selection. The most important underlying assumption of the quantitative genetic approach is that the traits being studied are under the control of not just one genetic locus with large, discrete allelic effects but rather the combined influences of many loci with smaller effects. The statistical procedures used are specifically designed to summarize the cumulative effects of all these loci simultaneously. Thus, quantitative genetic approaches are best suited to continuously-distributed characters rather than characters with categorical phenotypes. Quantitative traits can be divided into life-history traits such as survival, growth, and fecundity which are directly related to fitness; morphological traits such as the size and shape of various structures; behavioral traits such as activity cycles, parental care or aggression; physiological traits such as metabolism, oxygen consumption, locomotor abilities, and so on; or biochemical traits such as the levels or activities of specific enzymes or pathways. Regardless of the type of traits being studied however, if sufficient numbers of known relatives of the appropriate types are available for study, it is possible to statistically partition the overall variation in phenotypes into a variety of genetic and environmental components. Box 1 reviews these components of variation.

What's most important is to understand is that, whereas quantitative genetic variation is an indirect measure of the influence of unobserved genetic factors on phenotypic traits, this approach to quantifying genetic variation within and among populations has a direct connection with phenotypic characteristics, and as such can be directly interpreted in the context of natural selection, adaptation, and evolutionary potential—major determinants of long-term population viability (see, for example, Lande & Arnold 1983 and references therein).

Box 1. Components of Quantitative Variation.

Quantitative genetic variation can be partitioned in a variety of components. It is important, however, to appreciate that all of these components of variation are specific to the trait, population, and environmental conditions studied because different populations can have different numbers and frequencies of alleles at the loci that control a given phenotypic trait and the effects of these alleles can vary among environments.

Additive

Additive genetic variance is produced by the statistically independent (additive) effects on the phenotype of all of the alleles at all of the loci that determine the trait of interest. In this context "independent" and "additive" refer to allelic effects that do not depend upon or interact with either other alleles at the same locus (dominance) or with alleles at other loci (epistasis). This is the most important component of genetic variance from an evolutionary and conservation perspective because it is the only component that can be transmitted from parent to offspring. It is usually quantified as a proportion of the total phenotypic variance and referred to as the "heritability" of a trait.

Dominance

Dominance genetic variance is produced in diploid organisms by the interactive effects between the two alleles carried at each locus by a single individual. In the presence of dominance, the phenotype of an individual depends not directly upon the identities of the pair of alleles carried by an individual (additive effects), but also upon the way the specific alleles interact. An allele is completely dominant over another if the organism's phenotype is determined entirely by that allele. Dominance can also be incomplete, in which case, dominance is quantified as the difference between a heterozygote's phenotype and the mean of homozygous genotypes for each of its alleles. Because sexually reproducing organisms can only contribute one of their two alleles to their offspring, dominance effects are not transmitted from parents to offspring and are thus not heritable.

Epistatic

Epistatic genetic variance is also produced by interactions among alleles but at different loci rather than at the same locus. In the presence of epistasis, the effects of alleles at one locus on an individual's phenotype depend upon the "genetic background" at other loci. In effect, the phenotype is determined by the entire multilocus complex of alleles at the interacting loci which cannot be predicted from individual allelic effects. Epistatic effects are not heritable because only one allele at each locus can be transmitted from parent to offspring and because segregation and recombination shuffle multilocus genotypes during meiosis.

Environmental

Environmental variance is the component of phenotypic variance that cannot be attributed to genetic effects of any sort, and is presumed to result from subtle differences in environmental conditions at other stages of development or at spatial scales smaller than those studied. If experiments use a variety of sites or blocked experimental designs, environmental variation can be further partitioned into components within and among these units.

Genotype by Environment Interaction

The additive, dominance, and epistatic effects of alleles and multilocus genotypes can all potentially differ under different environmental conditions. As a result, alleles and allelic combinations that are favorable in one environmental can be less favorable or even detrimental in other environments. Genotype by environment interaction can promote population divergence if different genotypes are favored at different geographical locations.

Molecular Genetic Variation

In contrast to the more "classical" approaches mentioned earlier, modern DNA-level molecular markers can directly access genotypic-level information, but for most types of markers this information is difficult or impossible to connect to phenotypes and thus difficult to interpret in the context of natural selection and adaptation. There's a virtual alphabet soup of molecular genetic markers available, and each has its own technical virtues and pitfalls. Most of the markers available in nonmodel organisms, and nearly all of the markers used to analyze populations, however, are presumably "selectively neutral" in that the various states (alleles) at these markers have no phenotypic consequences and are thus effectively invisible to natural selection. The exceptions can be either sequence polymorphisms within actual genes or regions that regulate gene expression and neutral markers physically linked to them that "hitchhike." As a result, variation at neutral molecular marker loci is typically analyzed in a completely different but equally rich theoretical framework from quantitative genetic variation (Kimura 1968, Kimura 1983, King & Jukes 1969). Within this analytical framework, data on the numbers of alleles at marker loci and their frequencies within and among populations can provide estimates of the degree to which individuals within populations avoid or preferentially mate with relatives or neighbors (inbreeding *versus* outcrossing), what proportion of the population successfully breeds (effective population size), and how many individuals migrate among populations *versus* staying at home (gene flow). In addition these markers can be used to quantify spatial and temporal differentiation among populations because of nonselective forces and to detect the impacts of historical events such as population bottlenecks and admixture.

Are the Two Related?

Evaluating quantitative genetic variation typically requires either extensive pedigree information or complicated experiments involving controlled crosses, whereas molecular genetic variation can be assessed by collecting tissue samples in the field and applying the appropriate genotyping technology in the laboratory. Obviously, the latter is much easier, and as a consequence, a great deal more information on wild populations has been collected using genetic markers than through quantitative genetic analysis (Frankham 1995a, Haig & Avise 1996, Hard 1995). Unfortunately, despite the obvious differences between the two, it has been widely assumed that variation within and among populations at the molecular level provides a reasonable approximation for potentially adaptive quantitative genetic variation and that evidence of population differentiation at marker loci thus represents local adaptation (e.g., Allendorf & Leary 1986, Houle 1989, O'Brien et al. 1985, Soulé & Yang 1973). Recently, however, a number of researchers have advocated testing this assumption, calling into question the utility of using selectively neutral molecular markers as proxies for adaptive genetic variation that contributes to the adaptation, persistence, and viability of populations (Crandall et al. 2000, Frankham 1999, Lynch 1996, Pearman 2001, Pfrender et al. 2000, Storfer 1996). In one of the most comprehensive empirical studies available, Reed and Frankham (2001) conducted a meta-analysis of the available literature, using all of the available studies that included estimates of

quantitative and molecular genetic variation within populations and asked if the two are correlated. Their results are enlightening, albeit discouraging. They found that "At best, molecular measures only explain 4% of the variation in quantitative traits. Most disturbingly, the relationship is weakest for the measures of greatest interest to evolutionary and conservation biologists, those associated with life history traits and heritabilities" (Reed & Frankham 2001).

Genetic Composition of Stable Populations

Whereas the focus of this paper is on how human activities such as restoration efforts can impact the genetic composition of populations, these changes are best understood as comparisons to an idealized population at what is typically referred to as Hardy-Weinberg equilibrium (see Conner & Hartl 2004). The Hardy-Weinberg Principle is quite simple, and refers to the fact that in the absence of mutation, selection, and migration, the genotype frequencies within a randomly mating population of infinite size can be calculated directly from allele frequencies. Biologically, this boils down to saying that if there are no systematic forces acting to change a population's genetic composition, and if the population is large enough to be free of stochastic fluctuations, then random mating among individuals is equivalent to the random union of gametes. As a result, at any one genetic locus, the frequency of a specific diploid genotype is simply the product of the frequencies of the two alleles it contains (i.e., their "encounter frequency") assuming that they are randomly distributed in time and space. Taking this a step further, if genetic loci behave independently, the same reasoning can be applied to multilocus genotypes, a situation termed "linkage equilibrium" or "gametic phase equilibrium." In this case, the frequency of any particular multilocus genotype can be calculated by multiplying together the frequencies of the single-locus genotypes it contains.

Mechanisms of Genetic Change

One definition of evolution is simply any genetic change over time, and it is important to realize that these changes need not be adaptive. Genetic change can occur when any of the conditions that lead to Hardy-Weinberg equilibrium are not met and migration, genetic drift, mutation, nonrandom mating, or natural selection acts on a population (Box 2). In this section, we will briefly address how each of these mechanisms can change the genetic composition of populations.

Box 2. Mechanisms of Genetic Change.

Mutation

Mutation refers to any change in the genome of an organism outside of the normal Mendelian processes of segregation and recombination that result from meiosis and sexual reproduction. Mutations can be as minor as a change in the identity of a single nucleotide or as severe as large-scale duplications, insertions and deletions, even of entire chromosomes or sets of chromosomes. Mutation is generally thought to be a random process that occurs very infrequently and has mainly deleterious effects, but rare favorable mutations are the ultimate source of all adaptive genetic variation.

Genetic Drift

A random genetic change in allele frequencies caused by sampling effects at low population size is referred to as genetic drift. Genetic drift occurs within populations but can contribute to divergence between populations if allele frequencies drift in different directions. Given sufficient time, genetic drift is expected to result in the fixation of a single allele at each locus. All alleles have a nonzero probability of drifting to a frequency of zero, but this probability is higher for rare alleles.

Migration/Gene Flow

Migration refers to the movement of individuals and the genes that they carry among populations or subpopulations. Depending on the level of migration among populations, they can range from sufficiently isolated to have entirely independent genetic dynamics to sufficiently connected to behave as a single large population.

Non-random Mating

Inbreeding, inbreeding avoidance, and spatially patterned systems of mating and dispersal all alter the probabilities that a given pair of individuals will mate and thus produce departures from Hardy-Weinberg equilibrium (i.e., an overabundance of either homo- or heterozygotes.

Natural Selection

Natural selection occurs when (1) individuals within a population have different phenotypes, (2) these differences in phenotypes are associated with differences in survival and/or reproduction. If the phenotypes under selection have a genetic basis, the result of natural selection is adaptive change in phenotype and in the allelic and genotypic frequencies at the loci that control those phenotypes. If the phenotypes are not genetically determined, natural selection has no genetic or phenotypic effects on the population.

Mutation

Although mutation is ultimately the source of all genetic variation, it is usually considered a weak force on ecological time scales because mutations are rare (typically 10^{-4} to 10^{-6} mutations per gene per generation). However, evidence is accumulating that in some oyster species, mutation rates may be much higher than in model organisms such as flies, mice, and humans either caused by higher rates of mutation per cell division or the large numbers of cell divisions required to produce 10–100 million eggs and many billions of sperm annually (Bierne et al. 1998, Hedgecock et al. 2004). Because the most mutations are deleterious, high rates of mutation are expected to result in reduced fitness or even in "mutational meltdowns" leading to local extinction in small populations (Lynch et al. 1995a, Lynch et al. 1995b). Paradoxically, however, oysters and other highly fecund species may be fundamentally different from other organisms. High rates of mutations may actually be an evolutionary advantage if unpredictable and highly variable recruitment places a premium on generating variable offspring (Hedgecock et al. 2004, Williams 1975).

Genetic Drift

Technically, all finite populations are subject to some degree of random genetic drift because the number of reproductive propagules produced is always less than the number of multilocus genotypes that are possible. However, the effects of drift vary from very weak in extremely large populations to very strong in extremely small populations. As a stochastic process, genetic drift can alter the frequencies of alleles at selectively neutral marker loci and nonneutral loci that control quantitative traits (quantitative trait loci or QTL) and thus effect both molecular and quantitative

genetic variation. Genetic drift is also the most appropriate null hypothesis against which observed genetic change must be tested to be considered nonrandom. Only spatial and temporal patterns in molecular or quantitative genetic variation that significantly differ from those expected as a result of random sampling effects should be interpreted as evidence of nonrandom changes, for example, to selection or nonrandom mating.

Migration

Another cause of genetic change within populations is migration among populations. When individuals move, they bring their genes with them, making their new population a bit more similar to the one they left. Obviously, this is a matter of degree, but theoretical and empirical studies indicate that in the absence of selection, surprisingly few migrants (on the order of one successful immigrant per generation) are needed to effectively prevent populations from diverging (see Mills & Allendorf 1996). On the other hand, even very high rates of gene flow can be insufficient to prevent population divergence and local adaptation if selection is strong enough. For example, in blue mussel (*Mytilus edulis*) despite a lack of evidence for population differentiation at most protein allozymes studied and evidence for extensive gene flow among populations, there is strong local adaptation over surprisingly small spatial scales. Specifically, polymorphisms at the *Lap* locus show strong correlations with environmental salinity, and allozyme variation at this locus is correlated with the ability to maintain cell volume through osmotic balance and thus plays an important role in acclimation to salinity (Koehn et al. 1980, Koehn & Hilbish 1987 and references therein).

Intuitively, gene flow would seem to always oppose adaptation, but in theory, migrants can also distribute generally beneficial mutations from their natal population to other populations and thus facilitate global and local adaptive change. Sewall Wright developed the idea of a shifting balance between random genetic drift, natural selection, and gene flow in which gene flow is low enough to maintain locally-adapted multilocus genotypes but high enough to effectively distribute rare favorable mutations among populations (Provine 1986, Wright 1931, Wright 1988). In a nutshell, Wright argued that random genetic drift in small populations allows them to cross through valleys in an ''adaptive landscape'' of all possible genetic configurations. Natural selection then pushes populations uphill toward high-fitness adaptive peaks, and migration subsequently distributes beneficial mutations to other populations allowing all populations to eventually climb the highest peaks in the entire landscape. The result is that the evolutionary potential and fitness of all populations is maximized. It is still unclear, however, to what extent natural populations meet the necessary conditions.

Non-Random Mating

Genetic change can also result from nonrandom patterns of mating. As discussed earlier, a major assumption of the Hardy-Weinberg equilibrium model is that alleles and genotypes are randomly distributed in time and space and those individuals within a population mate at random. If behavior or unobserved physical barriers to migration violate this assumption, single and multilocus genotype frequencies cannot be predicted from allele frequencies without additional information. Behavioral mechanisms and demographic or geographic factors can lead to either preferential mating among individuals with similar genotypes (inbreeding) or inbreeding avoidance. The former results in an over-abundance of homozygous genotypes and the latter an overabundance of heterozygotes relative to expectations that, in the absence of selection, results in no change in allele frequencies. Neutral molecular genetic variation is expected to follow this pattern. If, however, homo- or heterozygotes enjoy higher fitness (i.e., selection is acting), then nonrandom mating can also have effects on the rate of change in allele frequencies and can alter both the mean and variance of fitness and other quantitative genetic traits. As well, if individuals mate nonrandomly with respect to heritable phenotypes, quantitative genetic variation will also be affected. The specific consequences of assortative mating are complex, but it can be generalized that if individuals preferentially mate according to phenotypic similarity (positive assortative mating), it will normally increase quantitative genetic variance and that negative assortative mating generally has the opposite effect (Conner & Hartl 2004).

Natural Selection

Natural selection is the dominant mechanism generating adaptation to the biotic and abiotic environment and, therefore the primary determinant of population persistence in the face of environmental change. It is important to note, however, that natural selection directly impacts only phenotypes. Gene sequences and genotype frequencies are only indirectly affected by natural selection and only to the extent to which they control phenotypes. Even if selection on phenotypic variation is strong, unless phenotypes are at least to some degree genetically determined, there will be no response to selection in terms of either phenotypic or genetic-level change.

Natural selection can be examined in a variety of ways. Quantitative, statistical approaches that examine, for example, temporal changes in phenotypes or allele frequencies, phenotype/fitness correlations, or correlations between allele frequencies and environmental factors can reveal natural selection using only phenotypic or allele frequency data (Manly 1985 is a good resource). Alternatively, if sequence data are available, the sequences of individual ''candidate genes'' can be compared among populations or species for signals of selection (Hughes 1999). It is important to keep in mind, however, that even if it is possible in some instances to identify specific genes that determine adaptively important phenotypes (e.g., the earlier mentioned *Lap* locus in mussels) most of the molecular markers currently used to study population-level patterns (e.g., microsatellite DNA, RLFP, AFLP, RAPD) have no known function and are presumed to be selectively neutral. This, is, however, changing rapidly as sequence information becomes more available for more organisms including oysters (Hedgecock et al. 2005). Especially useful in this regard are large collections of expressed sequence tags (ESTs) and single nucleotide polymorphisms (SNPs) within them because these represent functional genes that may control traits under selection rather than random, selectively neutral polymorphisms.

Whereas there is a great deal of complex theory to model the effect of natural selection on populations, the most important relationship is simple: the expected single-generation change in the population's average phenotype is directly proportional to the trait's heritability and the strength of selection (Falconer &

Mackay 1996, Lande 1976). In general, (i.e., unless environmental conditions fluctuate unpredictably) populations with sufficient levels of adaptive genetic variation to respond to environmental changes are expected to produce individuals well-suited to new conditions and therefore to persist, whereas populations with insufficient adaptive genetic variation are expected to be eliminated by natural selection because of their inability to produce suitable phenotypes (Lande & Shannon 1996).

Characterizing Natural Populations

Measuring Quantitative Genetic Variance Within Populations

As mentioned briefly earlier, estimating quantitative genetic variability involves determining the degree to which phenotypic similarity between individuals reflects genotypic similarity and obviously requires both types of data on the same individuals. This estimation can be achieved in a number of ways. The most common approach is to bring animals collected in the field into the laboratory and produce a series of controlled matings. Progeny phenotypes are then evaluated either under laboratory conditions or by returning appropriately marked or caged offspring to the field. Analysis of variance approaches (Falconer & Mackay 1996, Lynch & Walsh 1998) or animal models (reviewed by Kruuk 2004) are used to estimate the components of variance within and among crosses, with the among-cross component representing genetic effects and the within-cross component representing environmental ''noise.'' The proportion of the total variance attributable to additive genetic effects is called the trait's heritability. The simplest experimental design consists of a large set of full-sib families (i.e., single-pair matings) in which each parent is used in only one cross and these families are tested in a single environment. Unfortunately, this approach has severe limitations. Full-sib experiments cannot partition additive genetic variance from dominance or epistatic genetic variance. Heritabilities estimated from full-sibs represent their combined effects and are termed broad-sense heritability estimates. Half-sib experiments in which individuals of one sex (typically males) are crossed with multiple mates, however, do allow partitioning of additive and nonadditive genetic effects because the alleles of the multiply-mated parent are paired with a variety of alleles in the other parents, and such experiments are capable of estimating the so-called narrow sense heritability or the proportion of total phenotypic variation attributable to strictly additive genetic variance. Further, experiments conducted in one environment cannot evaluate genotype-by-environment interactions, and multiple environments are necessary to address genotype-by-environment interaction. Another common and simple approach is to obtain measurements on both parents and offspring. In these experiments, the regression coefficient of the parental phenotype(s) on offspring phenotype is a direct estimate of the heritability, but unless environmental conditions can be tightly controlled, problems arise because the parents and offspring cannot usually be evaluated in the same environment. Quantitative geneticists, especially plant and animal breeders, have developed a staggering diversity of experimental designs that generate much more complex arrays of relatives and thus allow for much more complex statistical partitioning of genetic variance, but these are seldom used in context of conservation.

Another way to estimate the quantitative genetic variability within a population is to determine the genetic relationships among free-living individuals and use this information to tease apart the genetic and environmental components of variance. These relationships can be observed directly or reconstructed using highly polymorphic molecular markers. There are currently several estimation methods available to reveal the relatedness of individuals using molecular marker information when pedigrees are unavailable (for recent reviews see Blouin 2003, Oliehoek et al. 2006). In general, these methods fall into two categories: (1) methods of moments that use continuous measures of relatedness among individuals estimated from the number of shared alleles in a multilocus genotype (Kalinowski et al. 2006, Li et al. 1993, Lynch 1988, Queller & Goodnight 1989, Wang 2002) and (2) maximum-likelihood methods that use marker information to infer categorical relationships among individuals by assigning them to families (Fernandez & Toro 2006, Mousseau et al. 1998, Smith et al. 2001, Thomas 2002). Analagous to these two methods of reconstructing relatedness, there are two methods for estimating quantitative genetic parameters from molecular marker data and phenotypic data on the same individuals: (1) regression methods that estimate the level of association between continuous measures of genetic relatedness and phenotypic similarity (Lynch 1988, Mousseau et al. 1998, Ritland 1996) and (2) likelihood methods that partition the variance components attributable to categorical family membership (Mousseau et al. 1998, Thomas et al. 2000).

Measuring Molecular Genetic Variance Within Populations

Molecular genetic diversity is much simpler to quantify because the required data can be obtained directly from field-collected samples. The most fundamental measure of molecular genetic variation is allelic richness, which is simply the number of different variants, or alleles, observed at a locus within a population. If there are many possible alleles, the number of individuals sampled will affect the number of alleles detected. To compare allelic richness among populations, it is necessary to correct for different sample sizes (Leberg 2002). If the genetically effective population size becomes constrained, this may be reflected in a reduction in allelic richness over time through genetic drift and loss of rare alleles. Allelic richness, however, does not provide any indication of the relative abundance or frequency of different alleles, and therefore ignores a great deal of information.

Another common metric used to quantify molecular genetic diversity that indirectly incorporates the relative abundance of alleles is heterozygosity, or gene diversity. Expected heterozygosity (*He*), refers to the proportion of heterozygotes expected under Hardy-Weinberg equilibrium given the number and frequency of individual alleles in the whole population. Observed heterozygosity (*Ho*) refers to the observed proportion of diploid individuals with two different alleles. Populations in which a small number of alleles occur at high frequency will be, on average, less heterozygous (more homozygous) than populations in which there is a more even distribution of allele frequencies and/or a larger number of segregating alleles. Calculations of observed and expected heterozygosity lead to a third approach, the partitioning of heterozygosity among hierarchical levels of organization ranging from subpopulations to interconnected sets of populations. As with quantitative genetic variation, there are numerous analytical approaches for this (Pearse & Crandall 2004), but the most widely used is the

series of hierarchical F-statistics developed by Sewall Wright (see Box 3).

Wright's F-statistics, also called fixation indices, are essentially deviations of observed genotypic frequencies from their expectations under Hardy-Weinberg equilibrium. Recall that at equilibrium the expected frequencies of hetero- and homozygous genotypes are simple functions of allele frequencies, and the allele frequencies themselves are expected to be equal in all subpopulations except for stochastic sampling effects. Statistically significant deviations from these expectations provide evidence that one or more of the assumptions of the null model are not met. F_{IS} is used to characterize within-population molecular genetic diversity whereas F_{ST} is used to characterize among-population molecular genetic diversity and will be discussed below. The sign and magnitude of the difference between observed and expected heterozygosity within a population can be interpreted as the consequences of nonrandom mating within populations.

Box 3. F-statistics Used to Partition Molecular Genetic Variation.

Wright's F-statistics are a set of hierarchical descriptors of how molecular-level allelic variation is distributed within and among populations and subpopulations. The basic idea is that in a randomly mating or panmictic population, with no mutation or selection, the proportion of heterozygotes and homozygotes at each locus should be simple probabilistic functions of allele frequencies (observed heterozygosity equals expected heterozygosity). Localized mating (i.e., limited migration), nonrandom mating (inbreeding or outcrossing), genetic drift, and natural selection all lead to deviations from the simple predictions. The F values described below represent the deviations that occur within subpopulations (F_{IS}), among subpopulations (F_{ST}), or within the population as a whole (F_{IT}).

F_{IS} compares the amount of inbreeding in a group to that of their subpopulation, and represents the deviation between the expected and observed proportion of heterozygotes within a single population or locality with no apparent barriers to migration. F_{IS} can range between –1 and 1; positive values of F_{IS} indicate a deficiency of heterozygotes, which in the absence of mutation and selection is an indication of inbreeding (i.e., individuals mate more often than expected by chance with relatives) or of unrecognized barriers to migration (population substructure). Negative values of F_{IS} indicate a deficiency of homozyogotes relative to random expectations produced by either the avoidance of matings between relatives or reduced survival of inbred progeny.

F_{ST} weighs the lowest population level (subpopulation) against the highest level (total). A deviation between the proportions of heterozygotes within local populations compared with random expectations, under the assumption that there are no geographical or behavioral barriers to migration and gene flow among local populations, indicates population substructure. F_{ST} ranges between 0 and 1 (F_{ST} can, in theory, be negative indicating a heterozygote excess caused by individuals preferentially mating with members of other populations (i.e., hyper-migration), but in practice this is never observed); an F_{ST} of zero indicates that the same allele frequencies occur in both populations, and an F_{ST} of one indicates completely different sets of alleles with no overlap. Positive values of F_{ST} indicate genetic divergence among subpopulations, the degree to which migration among subpopulations is constrained, and that individuals have a higher probability of mating with members of their own local population than with members of other populations. Thus, F_{ST} can be interpreted as a measure of the degree of reproductive isolation between subpopulations and of the level of molecular genetic differentiation between them.

F_{IT} indicates the relative amount of inbreeding in a group of individuals to the population as a whole, and can be thought of as the overall deficiency of heterozygotes from both mating among relatives and population level effects combined.

Testing for Inbreeding and Inbreeding Depression

The hierarchical F-statistics referred to earlier in the text can also be thought of as different types of "inbreeding coefficients" that differ only in terms of the randomly mating reference population to which they are compared. As a result, the term "inbreeding" can actually refer to several related phenomena with similar genetic consequences (increased homozygosity) that differ in how they occur and how they are measured (reviewed by Keller & Waller 2002). The term "inbreeding" is typically used to refer to a specific form of nonrandom mating within populations, more precisely "pedigree inbreeding"—the probability that the two alleles of a diploid individual can be traced to a single ancestor. This probability (F) can only be estimated relative to a specific ancestral generation beyond which no pedigree information is available. The population-level mean of F is F_{IT} . A second mechanism of within-population inbreeding is through nonrandom mating in the absence of spatial subdivision and is measured as the degree of relatedness between actual mates relative to random expectations. By this definition, "inbred" individuals result from matings between parents more closely related than the average random pair of individuals in the extant population. This form of within-population inbreeding is quantified at the population-level by F_{IS}, with inbreeding indicated by a deficiency of heterozygote genotypes relative to expectations under Hardy-Weinberg equilibrium, and is estimated from allelic and genotypic frequencies rather than pedigree information. Whereas the distinction may seem obscure, it has important implications. For example, in populations with small census or effective population size, there can be considerable pedigree inbreeding (F_{IT}) even under random mating ($F_{IS} = 0$) because of the high frequency of relatives in the population. A third mechanism producing inbreeding is nonrandom mating caused by spatial subdivision leading to among-population inbreeding. Under this scenario, even if mating is random within semi-isolated subpopulations, the probability of matings between relatives is higher than that expected under random mating in the total population. This form of inbreeding is quantified at the molecular level using F_{ST}.

The simplest approach to studying within-population inbreeding depression uses pedigree records of natural or controlled matings to unambiguously determine individual-level values of F relative to some reference generation and to ask if there is a relationship between individual-level F and fitness or its component traits, and this approach has been used in several studies of inbreeding in oysters (Beattie et al. 1987, Bucklin 2002, Evans et al. 2004b, Longwell & Stiles 1973, Mallet & Haley 1983). When such pedigree information is unavailable, within-population inbreeding can also be studied using molecular markers. Traditionally, multilocus heterozygosity (MLH) has been used as a proxy for individual-level inbreeding, and a number of studies have found significant correlations between multilocus heterozygosity and fitness in a wide range of taxa (for reviews see Avise 1994, Britten 1997,

David 1998, Hansson & Westerberg 2002, Mitton 1993, Roff 1997).

A slightly different approach is to use molecular marker genotypes to estimate parental relatedness for known mating pairs rather than to compare alleles within individuals of unknown parentage. Parent-offspring relationships can be unambiguously identified through single-generation pedigrees, controlled matings, or molecular marker based assignment techniques (for recent reviews see Blouin 2003, Oliehoek et al. 2006), and combining this information with estimates of the relatedness of breeding pairs provides information on the levels of inbreeding depression in their offspring (e.g., Camara et al. 2008).

Under population subdivision (or in the extreme case independent small populations), genetic drift can lead to high frequencies of different deleterious alleles in different (sub)-populations, reducing the fitness of entire (sub)populations, a phenomenon best termed among-population inbreeding depression. Because all of the individuals within such (sub)-populations are similarly homozygous, matings among known relatives within them may not increase homozygosity substantially and, therefore, produce no detectable within-population inbreeding depression. Thus, among-population inbreeding depression can only be detected by examining crosses among (sub)populations that increase heterozygosity. Within- and among-population inbreeding can have important implications for oyster restoration efforts, and both can arise in populations with small effective population sizes.

Estimating Effective Population Size

From a genetic perspective, the effective population size is much more important than the actual number of individuals in a population. The concept of effective population size (N_e) is an abstraction that has very real implications. N_e represents the size of an idealized population (i.e., 1:1 sex ratio, random-mating, no immigration or emigration, no selection) that would result in the same level of the characteristic in question as that observed in the population under study. Effective population size can be estimated using either demographic or genetic parameters; we focus here on genetic estimators. Because there are a variety of population-level characteristics that might be of interest, there are also a variety of effective population size estimators (inbreeding effective size, variance effective size, eigen value effective size, mutation effective size and coalescent effective size). The two presented in Box 4, however, are the most widely used. Inbreeding effective population size refers to the rate of inbreeding, and variance effective size refers to rate of genetic drift (Ryman 1994). Franklin (1980) suggested an inbreeding effective size of at least 50 to guard against inbreeding, although variance effective population sizes of up to 5,000 may be necessary to avoid losses of variability (Lande & Barrowclough 1987). There are varied methods for estimating both types of N_e from genetic information. For example, neutral genetic marker data can be used to estimate N_e based on variance in allele frequencies between years (e.g., Waples 1989) or gametic disequilibrium within years (Bartley et al. 1992). Wang and Whitlock (2003) argue that the assumption of no migration is often untenable and that migration biases estimates of N_e. To solve this problem, they developed an approach to jointly estimating both effective population size and migration rate using samples over both space and time.

Box 4. Types of Effective Population Size (N_e).

Effective population size, in an ideal population with random mating, no mutation, nonoverlapping generations, and no selection, is equal to the census population size. In most real populations, however, the effective population size is a fraction of the census size. Whereas effective population size can be estimated using demographic parameters such as sex ratio and family size, genetic estimators generally provide greater accuracy. In many situations where populations are stable, the values for the two N_e estimators described here are similar; they can, however, differ significantly when populations experience rapid increase or decrease in census numbers. For example, large N_{eI} and small N_{eV} are indicative of recent declines in census size (bottleneck), whereas small N_{eI} and large N_{eV} are evidence of recent population increase.

Inbreeding (N_{eI})

Inbreeding effective population size is the size of an ideal population that would show the same generation-to-generation change in the average level of inbreeding (i.e., increase in homozygosity) as that observed in the population being studied. As an indicator of relatedness in the population, the inbreeding effective size responds more slowly to changes in the census population size, thus providing a valuable retrospective. The level of relatedness in a population changes much more slowly than changes caused by genetic drift.

Variance (N_{eV})

Variance effective population size is the size of an ideal population that would show the same random generation-to-generation variation in allele frequencies (i.e., genetic drift) as that observed in the population being studied. Changes in allele frequencies between generations can provide robust estimates of the variance effective size, especially in smaller populations. The variance effective population size is an indicator of genetic drift, decreases rapidly in response to population declines and conversely increases quickly with population growth.

It is important to point out that effective population sizes are typically smaller and sometimes much smaller than the census population size (In rare cases, if variance in reproductive success is smaller than random expectations, effective population size can be larger than the actual population size). Both allelic diversity and heterozygosity can be low in populations of small effective population size, even when the census population size is large. Factors contributing to low effective population size include large fluctuations in census population size that produce genetic ''bottlenecks,'' high variance in reproductive success among individuals, skewed sex ratios, high genetic load, stochastic environmental variation, small-scale spatial structure or other factors that result in nonrandom mating. In shellfish hatcheries, these factors may be exacerbated by the high fecundity of many bivalves and standard hatchery practices geared towards producing large quantities of seed, sometimes from small numbers of parents (e.g., Dillon & Manzi 1993, Newkirk 1978).

Quantifying Gene Flow and Migration Among Populations

Neutral molecular genetic markers are well suited to estimating gene flow and migration. As stated earlier, the most common metric of population differentiation is F_{ST} (Box 3) and closely-related variations, although there is now an extensive list of alternative approaches that can estimate not only population differentiation but also a number of other parameters (Pearse &

Crandall 2004). What these approaches all have in common, however, is that they use presumably neutral molecular marker data collected from a number of populations, and extract information on the current and/or historical connectivity among them. F_{ST}—like methods begin with researcher-defined populations, and produce estimates of differentiation on the assumption that they are correctly defined. An alternative approach, however, is to essentially turn the question around, and apply genotypic clustering procedures to identify populations from the data (Pritchard et al. 2000).

Typically, the number of successful migrants per generation is estimated from molecular genetic data collected from a number of potentially interconnected (sub)populations and analyzed under the assumption of selective neutrality. A variety of statistical approaches can be used. One method uses the average frequency of "private alleles"—alleles that occur in only one of the subpopulations (Slatkin 1985). According to theory, the logarithm of the average number of migrants per generation (N_m) is an inverse linear function of the average frequency of private alleles. Other approaches use the relationship $F_{ST} = 1/(4N_e\mathrm{m} + 1)$, where m is the immigration rate (see Slatkin 1987), but this method requires potentially unrealistic assumptions (Whitlock & McCauley 1999). Other approaches use isolation-by-distance methods (reviewed by Rousset 2001), coalescence theory (Nielsen & Wakeley 2001), migration matrix likelihood models (Beerli & Felsenstein 2001) and assignment methods (Rannala & Mountan 1997). Wang and Whitlock (2003) argue for joint estimation of effective population size and migration rate, which requires sampling both temporal and spatial sampling.

Testing for Adaptive Differentiation and Local Adaptation

For neutral genetic markers with no phenotypic consequences, natural selection is not a factor, and genetic drift, nonrandom mating, and gene flow are sufficient to account for both within-population dynamics and differentiation among populations. Population differentiation for quantitative traits and at the loci that contribute to them (QTL), however is also a product of natural selection (Le Corre & Kremer 2003). Further, apparent panmixia with respect to neutral molecular markers can mask adaptive variation present among populations (Utter 2004), and the correlation between quantitative and molecular markers is weakest for life history traits (Reed & Frankham 2001). Genetic differences produced by natural selection, therefore cannot be examined using neutral markers and are best measured with quantitative genetic approaches (Reed & Frankham 2001, Storfer 1996).

The simplest way to test for local adaptation is to perform reciprocal transplant experiments using large, random samples of genotypes from each population. Assuming that experiments are properly designed to eliminate population-specific environmental effects (e.g., by spawning all populations in one hatchery facility), if local stocks perform better than transplanted ones, it is reasonable to conclude that the overall differences reflect genetic effects and that the populations are locally adapted. This approach, however, can be problematic if political or management policies preclude transplantation.

A more comprehensive approach would be to estimate not only the overall differences in performance among populations, but simultaneously the levels of quantitative genetic variation within and among populations. Measuring quantitative genetic differentiation and testing for local adaptation is a straightforward extension of the approaches described earlier for estimating quantitative genetic variation within populations. By incorporating multiple populations and applying an F_{ST} -like approach, a statistic termed Q_{ST}. can be calculated to partition not heterozygosity but rather quantitative genetic variation into, within, and among-population components (Spitze 1993). Because genetic drift can also produce quantitative genetic differentiation among populations (Lande 1992), an appropriate test for local adaptation is to compare Q_{ST} for specific phenotypic traits to F_{ST} calculated from neutral molecular markers. For any given trait, if the quantitative genetic variation among populations is a product of genetic drift, Q_{ST} is expected to be equal to F_{ST}, whereas if Q_{ST} exceeds F_{ST}, that character is likely to be involved in local adaptation (McKay & Latta 2002, Merilä & Crnokrak 2001, Spitze 1993). This is an active area of research, however, and appropriate statistical approaches are still being developed (e.g., Goudet & Buchi 2006, Latta 2004, O'Hara & Merilä 2005, Porcher et al. 2006, Whitlock 2008).

Another approach would be to compare F_{ST} calculated for markers known to be selectively neutral to F_{ST} calculated for markers with known functional consequences (i.e., "candidate genes") or markers tightly linked to QTL for ecologically important traits, but this requires a great deal of prior information and is currently out of reach for most nonmodel organisms (McKay & Latta 2002)

Unfortunately, because estimates of genetic variation measured in two populations experiencing two different environments include population-specific environmental components of variance that cannot be partitioned, the only way to separate the genetic and environmental effects is to not only incorporate information on the genetic relationships among individuals but to also use experimental designs that de-couple genotype-environment correlations. The most common experimental design is a nested analysis of variance with individuals nested within families within populations evaluated in a single environment or "common garden." Whereas it would also be highly desirable to conduct these experiments not in just a single environment but in multiple environments (preferably the actual environments experienced by the natural populations because genotype-by-environment interactions could cause genetic components of variance to vary among environments), this amounts to a reciprocal transplant experiment that also incorporates a mating design and is subject to the same potential political and management constraints as other reciprocal transplants.

Testing for Outbreeding Depression

Outbreeding depression refers to negative effects on the fitness of individuals derived from matings between genetically dissimilar individuals. Templeton (1986) describes two major causes of outbreeding depression, but for our purposes here it seems to us more appropriate to define three types based on the genetic mechanisms that produce them. Type 1 is a decrease in fitness of the hybrid even if the genetic basis of life-history traits is entirely additive. If for example, two environments require extreme phenotypes for survival and hybrids between populations adapted to these environments have intermediate phenotypes, hybrid progeny will be mal-adapted to both extremes. This can be thought of as a simple consequence of the number of

"good genes" for a population's home habitat being "diluted" by an influx of inferior genes. Type 2 is the opposite of heterosis or "hybrid vigor" and also results in maladapted phenotypes, but in this case because of the breakup of beneficial allelic combinations at individual loci (i.e., the masking of beneficial recessive alleles by an influx of inferior dominant alleles). Type 3 is more complex, and results from the disruption of favorable multilocus epistatic combinations through recombination with exogenous genomes. Type 1 and Type 2 outbreeding depression are expressed in the first generation of hybrids, whereas Type 3 outbreeding depression generally appears in the second or later generation of hybrids (Lynch & Walsh 1998). Whereas Type 1 outbreeding depression requires local adaptation, Types 2 and 3 do not. If, for example, two genetically isolated populations have high frequencies of different locally beneficial alleles that show dominance or different beneficial multilocus epistatic gene complexes as adaptations to similar environmental conditions, they may both be equally adapted to either location as long as they do not interbreed. If, however, these equally but differently adapted populations interbreed, outbreeding depression could still occur.

Testing for outbreeding depression is exactly analogous to testing for among-population inbreeding depression above, requiring an empirical approach that compares the performance of crosses among populations to crosses within populations (Hedrick & Kalinowski 2000, Keller & Waller 2002). Ideally, these crosses would include F_2 or more advanced intercrosses and be evaluated in the habitats from which the populations are derived as for tests of local adaptation. Indeed, the same experiment could be used for both purposes if this is feasible. Alternatively, common garden approaches could be used, though there is some risk that the results would be affected by genotype-by-environment interactions and thus not apply to the natural environments in which the populations are found.

POTENTIAL ANTHROPOGENIC IMPACTS ON THE GENETICS OF NATURAL POPULATIONS

A variety of human activities can alter the potential for, and course of, evolutionary change. In this section, we summarize these effects. In a later section, we consider which types of restoration activities may or may not produce or reverse them.

Reduced Population Size/Population Bottlenecks

Harvesting, pollution, and habitat destruction that permanently reduce the size of populations lower the census and effective population size and thus promote genetic drift and inbreeding. In addition, population bottlenecks, temporary reductions in population size, can also result in long-term losses of molecular genetic diversity. In essence, if a population is, at any point, reduced to a small number of individuals, some of its molecular genetic variation can be lost because the small number of individuals that passes through the bottleneck cannot capture the diversity of the larger population from which it is derived. Depending on the causes of the population bottleneck, the numbers of individuals in the population may recover rapidly, but the lost alleles can only be replaced by mutation or immigration from other populations. However, depending on the number and frequency distributions of the remaining alleles, heterozygosity may recover rapidly or not at all. Even large populations that have passed through a small bottleneck can have low N_e. In such populations, genetic drift can be strong and inbreeding can be intense if substantial proportions of the population are derived from a few common ancestors.

The effects of reductions in population size and bottlenecks on quantitative genetic variation, however, are potentially more complicated. In populations with little or no epistatic genetic variance, additive genetic variance is expected to behave similarly to molecular genetic variance. However, in populations with high levels of epistatic genetic variance, a reduction in the population size can "convert" epistatic genetic variance into additive genetic variance by reducing the levels of polymorphism at interacting loci (Bryant & Meffert 1988, Goodnight 1988).

Changes in Patterns of Gene Flow

Human activities frequently also alter the patterns of gene flow among populations either by breaking the connections between populations or subdividing large, continuous populations into smaller, isolated fragments. In either case, the result is a reduction in the levels of genetic exchange, increasing the potential for populations to accumulate differences through either genetic drift or local selection depending on the effective population sizes of the newly-isolated fragments. If fragmented populations are small, then they will be more susceptible to genetic drift and inbreeding. If they are large and if there are locality-specific conditions, then they may evolve local adaptations that could not occur in the presence of gene flow.

At the other end of the spectrum are human activities that increase gene flow through the translocation of individuals among populations that are naturally isolated from each other. Depending on the history of the populations, this human-mediated gene flow can either replace natural gene flow that was previously disrupted or produce unnatural gene flow among historically isolated populations. This can have positive effects such as increasing or restoring heterozygosity, decreasing within- and among-population inbreeding depression or delivering adaptive genetic variation. Alternatively, enhancing gene flow can also result in outbreeding depression in locally adapted populations when these adaptations depend upon the maintenance of epistatic gene complexes.

Variation in Reproductive Success

One of the major factors influencing effective population size in oysters is the variance in reproductive success (Hedrick 2005). If this variance is high (i.e., a small number of individuals make inordinately high contributions to the next generation), then the effective population size will be small relative to the census population size. Human activities can elevate this variance by either artificially depressing or enhancing the reproductive contribution of a subset of the potentially reproductive population. Either way, N_e is reduced. Of course, the consequences of reducing N_e depend on the magnitude of the effects. For example, supplementing small populations with large numbers of progeny derived from few parents can reduce N_e dramatically, whereas supplementing large populations with modest numbers of animals derived from many parents would have a trivial impact (see Hedgecock & Coykendall 2007 for an excellent review).

Artificial Selection/Domestication Selection

Selection in the hatchery, whether deliberate or inadvertent, will result in genetic differences between hatchery and wild populations unless broodstock management techniques are used to reduce inadvertent "domestication selection." First, regular broodstock procurement from the wild can aid in the randomization of genotypes and reduce the population's exposure to the hatchery environment. Second, equalizing family sizes has been shown to maintain higher levels of reproductive fitness (Borlase et al. 1993) and reduce domestication selection (Allendorf 1993). It is worth noting that domestication selection is deleterious only in the restoration/supplementation context at hand. Adaptation to the hatchery environment is a very real benefit for commercial aquaculture in that it can increase larval survival under hatchery conditions and thus the profitability of hatchery-based seed production. Whereas it is tempting to view this as beneficial to restoration efforts as well, and it is undeniable that enhanced larval survival in the hatchery directly translates into larger numbers of seed oysters for use in population supplementation efforts, it cannot be simply assumed that these advantages also apply to natural reproduction in the wild where larvae face a wide array of challenges that are deliberately eliminated in the hatchery. It is important, therefore, that commercially cultured and wild populations remain distinct.

Examples

Effects of Cultured Finfish on Wild Populations

Whereas there are very few instances in which shellfish restoration efforts have included sufficient genetic monitoring to document the potential effects we described earlier, there are a number of documented cases of the genetic effects of aquaculture on conspecific wild populations of finfish. A striking example is from Thailand, where a restoration project focused on the endangered Mekong giant catfish (*Pangasianodon gigas*) released 10,000 hatchery-reared fingerlings in 2001. Parentage analyses indicated that 95% of the released fingerlings were from the same two parents (Hogan et al. 2004, Na-Nakorn et al. 2006). Stock enhancement of the Japanese flounder *Paralichthys olivaceus* provides another example. Past efforts to increase population levels included breeding via mass spawning in mesocosms; a large variance in reproductive success reduced N_e by 80% from the already small hatchery census size (Sekino et al. 2003). In two recent studies, a majority of progeny were sired by a single male (99% in *Paralichthys olivaceus* (Sekino et al. 2003) and 55% in *Lates calcarifer,* (Frost et al. 2006). Osborne et al. (2006) found evidence of inbreeding in hatchery populations of the endangered Rio Grande silvery minnow, *Hybognathus amarus*. These losses of genetic variability may ultimately jeopardize survival of these species *via* a significant reduction of adaptive potential. The genetic effects of cultured-wild interactions in Pacific salmon provide other examples (Utter 1998, Waples & Do 1994). Numerous studies have evaluated the effect of farmed Atlantic salmon escapes on wild populations. Although most studies have demonstrated lower fitness of fish of farm origin (e.g. Araki et al. 2007, 2008), increasing numbers of escapees, combined with their lower overall fitness may result in accumulated fitness depression and increase the likelihood of extinction in vulnerable wild populations (McGinnity et al. 2003)

Effects of Cultured Invertebrates on Wild Effective Population Size

In marine invertebrates, a large variance in reproductive success has been hypothesized to constrain effective population size (Hedgecock 1994); for Pacific oysters it has been shown that hatchery N_e can be much lower than in wild populations (Gaffney et al. 1992, Hedgecock et al. 1992, Hedgecock & Sly 1990, Saavedra 1997). A number of studies have demonstrated high variance in family reproductive success in the hatchery environment, in mass spawns and in more controlled breeding programs. French researchers, for example, have studied family size variance in the Pacific oyster, *Crassostrea gigas*, (Boudry et al. 2002, Taris et al. 2007, Taris et al. 2006). These meticulous studies have found that gamete quality, sperm-egg interactions, and genotype dependent viability contribute to the large variance in reproductive success in *C. gigas*. Longwell and Stiles (1973) found evidence for gamete cross incompatibility in *C. virginica*. This phenomenon may exist because of a high number of recessive lethal mutations; Launey and Hedgecock (2001) calculated the "average" *C. gigas* individual carries 14 recessive lethal mutations, and this would certainly exacerbate variance in reproductive success such as that found in *C. gigas* (Li & Hedgecock 1998). If this effect is ubiquitous in other marine molluscs, it presents a significant challenge for general conservation of genetic diversity.

Among marine invertebrates, there have been few empirical estimates of effective population sizes in wild populations. Li and Hedgecock's (1998) study showing high variance in reproductive success in Pacific oyster provides indirect evidence of low effective population size relative to the census population. This study was conducted, however, in Dabob Bay, WA, where the Pacific oyster was introduced from Japan approximately 100 y ago. Natural spawning is known to occur in the inland waters of Washington, but mass spawns, historically, take place only sporadically and in isolated embayments. These relatively narrow environmental windows of opportunity for successful spawning and fertilization may limit the reproductive success of many, even most adults in this nonnative species. Native bivalves, on the other hand, may be well adapted to successful spawning in this environment. Gaffney et al. (1996) reported low effective population size estimates in a reseeded population of red abalone, *Haliotis rufescens* compared with a natural population. However, a subsequent reassessment by Burton and Tegner (2000) found no evidence of enhancement, and attribute the results of Gaffney et al. (1996) to either rapid rebound from a genetic bottleneck, or genotyping errors.

As mentioned earlier, a high variance in reproductive success creates a low N_e/N ratio in wild populations (Hedgecock 1994), and among long-lived marine species with high fecundities the N_e/N ratio in wild populations can be quite low. Hauser et al. (2002) observed a N_e/N ratio of 0.00001 in red snapper, and in red drum Turner et al.(1999) estimated the N_e/N ratio to be 0.004. Gomez-Uchida and Banks (2006) observed a N_e/N ratio of 0.0004 in darkblotched rockfish, and Herbinger et al. (1997) also found correspondingly low ratios in Atlantic cod. If a much higher N_e/N ratio is maintained in cultured populations, this may have neutral or even positive effects on genetic diversity in wild populations (Hedgecock & Coykendall 2007). For example, Hedrick et al. (2000) measured the three Ryman & Laikre (1991) parameters in a carefully managed Chinook salmon supplementation program, and found increased effective sizes in the total population as a result of hatchery supplementation.

Effects of Aquaculture on Wild Genetic Diversity

There are very few empirical examples of genetic effects of cultured marine invertebrates on wild conspecifics; the existing studies provide mixed results. Natsukari et al.(1993) detected significant genetic differences among wild, cultured, and mixed populations of the sea urchin *Pseudocentrotus depressus* in Japan using nine allozyme loci; the genetic differences (F_{ST} values) were highest in comparisons involving cultured stocks. In one cultured and one mixed population, significant reductions in expected heterozygosities (25% and 15%, respectively) were noted alongside a similar reduction in the proportion of polymorphic loci (27% and 9%, respectively). These seeding efforts may be compromising wild genetic variability if interbreeding occurs.

Luan, et al. (2006) found an F_{ST} of 0.023 between wild and cultured Kuruma prawn *Marsupenaeus japonicus* and a 35% loss of low-frequency microsatellite alleles in cultured versus wild populations. In two species of abalone, *Haliotis rubra* and *H. midae*, approximately 40% of relatively infrequent alleles present in wild collections were lost in cultured samples (Evans et al. 2004a). In addition, alleles relatively rare in the wild collections were often the most frequent in the cultured groups, and relatedness levels were high in two cultured groups.

Results of genetic assessments have, however, been mixed. Apte et al.(2003) used allozymes, mtDNA, and RAPDs in an attempt to detect effects of cultured Greenshell mussel (*Perna canaliculus*) on wild populations. There were no significant differences in observed heterozygosities between cultured and wild populations for allozymes. Using AFLP markers, no marked differences in observed heterozygosities were found between cultured and wild pearl oyster, *Pinctada fulcata* in southern China, although two of three cultured populations exhibited more fixed loci than proximate wild populations (Yu & Chu 2006). Similar assessments of selected strains versus wild *Crassostrea virginica* oysters, using microsatellite and AFLP data, revealed reduced allelic diversity in hatchery stocks (Carlsson et al. 2006, Yu & Guo 2004).

To detect possible introgression from cultured to wild populations of the hard clam *Mercenaria mercenaria*, Metzner-Roop (1994) used two allozyme locus *GPI* alleles present at high frequencies in cultured and rare in wild populations as a genetic marker. Despite repeated outplants of cultured stocks over the course of eight years, elevated frequencies of the marker alleles were not detected in collections of 300 individuals from four wild locations (Metzner-Roop 1994).

A RANGE OF RESTORATION OPTIONS WITH VARYING GENETIC IMPACTS

The five primary options available for oyster restoration, described later and listed in Box 5, have distinct advantages and disadvantages from both pragmatic and genetic perspectives. The most successful restoration efforts will likely be site-specific strategies that balance human and technical resources, ecological conditions, and genetic considerations for the persistence of the species once restoration efforts cease. We have ordered our discussion of these options according to their potential for negative genetic impacts, which range from inadvertent and minimal to intentional and severe.

Option 1: Do Nothing

Conceptually, the simplest route to the recovery of native oyster populations is to "do nothing" and allow natural forces to run their course. It is important, however, to emphasize this should be interpreted quite literally, Doing "nothing" does not mean simply perpetuating the *status quo* and ongoing negative impacts on oyster populations, but instead means attempting to eliminate most or all anthropogenic impacts on native oyster populations to facilitate natural recovery. Appropriate measures may include stopping recreational and commercial harvesting, eradicating introduced species that compete with and prey on native oysters, eliminating introduced diseases and parasites, as well a stopping pollution, contamination, dredging and filling. Clearly, "doing nothing" that impacts native oysters would require enormous changes in the ways humans interact with not merely oysters, but also with the estuarine ecosystems in which they live, and is much more complicated in practice than in principle.

From a genetic perspective, this approach would seem to have no unnatural impacts, and as a result generate no concerns. However, this assumes that current patterns of genetic variation within populations and gene flow among them are suitable for long-term persistence, which may or may not be the case. If, for example, native oyster populations were historically large, closely spaced, or even continuous, and thus connected by extensive gene flow, remnant contemporary fragments may suffer from low genetic diversity and high levels of inbreeding. These problems cannot be corrected unless gene flow is re-established *via* either restoring natural connectivity among populations or artificially transplanting animals.

Option 2: Habitat Restoration

Because it may be impossible to completely eliminate some anthropogenic impacts such as pollution and dredging, and because others (e.g., siltation, introduced species) will not simply reverse themselves even if current human impacts are eliminated (Mann & Powell 2007), restoring or augmenting the availability of suitable habitat could provide substantial benefits. This "If you build it they will come" approach typically takes the form of adding suitable hard substrate to areas that either currently or formerly supported native oyster populations, and relies on several assumptions: (1) that appropriate substrate for larval settlement is currently a limiting resource, (2) that existing remnant populations produce sufficient larvae to populate additional substrate, (3) that there are no barriers to larval dispersal that would prevent restored substrate from being colonized, and (4) that there are no biotic or abiotic factors that would prevent colonizing larvae from growing into reproductively mature adults. From a genetic perspective, there are no unnatural genetic effects predicted from habitat restoration efforts. However, as with the "do nothing" option above, if natural populations historically connected by gene flow are now isolated because of anthropogenic fragmentation, restoring habitat will not restore natural levels of gene flow unless careful consideration is given to the specific locations targeted for habitat restoration. Ideal locations would (a) currently receive abundant larvae from the existing configuration of remnant populations and (b) once populated, provide abundant larvae to other existing populations or areas targeted for future habitat restoration efforts.

Box 5. Summary of Available Restoration Strategies.

Option	Pros	Cons	Information Needed	Actions	Relative Cost
Do Nothing	"Natural" processes restore populations; unlikely to perturb genetic population structure	Assumes source populations exist, genetic variability is sufficient, existing connectivity/gene flow is representative of historical state; fragmented populations may lack genetic diversity and fail to reconnect; potentially slow response	Historical habitat structure and locations; nature and extent of anthropogenic impacts	Eliminate: recreational and commercial harvest, introduced species that may interact including disease and parasitic organisms, pollution, contamination, dredging, filling	Low to exorbitant depending on extent of local anthropogenic impacts
Habitat Restoration	Unlikely to artificially change genetic population structure.	Assumes populations are habitat-limited and that source populations exist; fragmented populations may not reconnect; assumes no barriers to larval dispersal; potentially slow response	Historical habitat structure and locations; source-sink dynamics of existing remnant populations; potential for restored sites to function as source populations	Place suitable hard substrate in impacted locales that may be habitat-limited	Low to Medium
Population Supplementation/ Reintroduction via transplants	Spatfall reflects natural reproduction at locale. If conducted on appropriate spatial scale using appropriate breeding stocks, unlikely to perturb genetic population structure or local adaptation.	May disrupt natural patterns of gene flow if conducted on wrong spatial scale; may impact adaptation on local scale.	Spatial scales and patterns of gene flow and local adaptations, or a planned stepwise approach to distribution.	Deploy suitable substrate within existing populations to collect spat, redistribute to restoration sites once colonized.	Low to Medium
Population Supplementation/ Reintroduction via hatchery production	Fast response, can quickly overcome lack of significant source populations or repopulate extirpated locales	Requires careful genetic management to avoid a) disrupting genetic population structure, b) inbreeding depression, c) outbreeding depression, d) reducing effective population size, e) domestication selection	Natural and hatchery effective population sizes, patterns of gene flow, adaptive differences among subpopulations.	Maintain high genetic diversity in hatchery outplants; 1:1 sex ratios, minimize family size variance; minimize kinship in matings; minimize hatchery effects	Medium to High

continued on next page

Box 5. Summary of Available Restoration Strategies.

continued

Option	Pros	Cons	Information Needed	Actions	Relative Cost
Genetic Rehabilitation via selective breeding	May overcome population size depression via genetic "improvement"	Selected traits may be maladaptive in the natural environment or may result in maladaptive responses in other traits caused by linkage disequilibrium with detrimental genes or pleioptropy; genetic diversity may be significantly reduced; Inbreeding depression may arise; less future adaptive potential in population; outbreeding depression may arise through interbreeding with wild stocks.	Effects of selection on genetic diversity, adaptive traits, G × E interactions, correlated traits, contributions of additive versus nonadditive effects; consequences of outcrossing with wild genotypes.	Establish an effective selective breeding program; maintain high genetic diversity in hatchery outplants; 1:1 sex ratios, minimize family size variance; minimize kinship in matings; minimize inadvertent hatchery effects; study genetic basis of targeted traits, correlation structure with other characters, and effects of outcrossing with wild population	Extremely High

Option 3: Redistribution of Natural Recruitment

In some instances natural recruitment may simply be inadequate to establish viable populations, even with habitat augmentation efforts. There may be no nearby populations capable of supplying larvae, fouling organisms may quickly dominate newly restored substrate and prevent larvae from settling, or the survival of newly settled juveniles may be low because of predation or competition. Under these circumstances, an attractive option may be to establish new or supplement existing but struggling populations with adults or juveniles that have already cleared these ecological hurdles. Supplementation can take several forms, described below, depending on where and how the supplementary animals are produced or procured. When supplementation is either (a) into extant populations that are not substrate-limited or (b) into restored habitats, these strategies can circumvent barriers to initial recruitment at restoration sites. However, for long-term success (i.e., population persistence) these strategies assume that (1) transplanted individuals will survive and reproduce, (2) either recruitment is local or the restored population will receive larvae from elsewhere, and (3) larvae produced will be able to settle, grow, and reproduce.

One possible supplementation strategy would be to either (a) collect and redistribute adults, or (b) temporarily deploy suitable settlement substrate within existing populations and transplant spat to restoration sites. From a genetic perspective, wild adult or spat collection and redistribution has less potential for unnatural genetic impacts than hatchery production (see later). Assuming that sufficient numbers of adults or juveniles are collected, their genetic composition necessarily directly reflects natural recruitment at the local scale. The only potential genetic impacts are those that may result from alteration of natural patterns of gene flow or the disruption of local adaptation.

Option 4: Hatchery Supplementation Using Wild Broodstock

A more aggressive form of population supplementation is to propagate large numbers of juveniles in hatcheries for transplantation into restoration sites. Hatchery propagation is similar to redistribution (earlier in text), and has the same potential to impact gene flow and local adaptation. It is important to note, however, that hatchery-based supplementation can result in further genetic impacts depending on the source and management of broodstock. In many respects, a hatchery population (especially a closed one) is analogous to a small wild population. The mechanisms of genetic change in wild populations (Box 1) also affect the genetic makeup of the hatchery population. Bivalve hatcheries, by virtue of their ability to exploit the high fecundity of individuals, can produce large numbers of progeny from a small number of parents, intensifying the effects of genetic drift and increasing the likelihood of inbreeding. Providing this sort of "reproductive assistance" to a small subset of the natural breeding population increases the variance in reproductive success, even if only slightly, and reduces effective population size. Proper broodstock and hatchery management actions can minimize genetic drift and inbreeding.

A more difficult problem, however, is the risk that the artificial hatchery and nursery environments in which larval and juvenile stages are propagated imposes inadvertent domestication selection on these early life history stages. Whereas it

may be seen as desirable to improve larval and juvenile survival in the hatchery, the phenotypes produced may or may not prove to be adaptive under natural conditions.

Option 5: Hatchery Supplementation Using Genetically Improved Broodstock

The restoration options described earlier presume that extant populations either contain sufficiently adapted genotypes to rebuild or that they harbor sufficient adaptive genetic variation to adapt over time. If this is in doubt, and if the specific traits preventing population expansion are well understood, an available option is the production or amplification of favorable genotypes through selective breeding followed by their intentional introgression into wild populations. Such ''genetic rehabilitation'' has been proposed as a viable strategy for restoring native *C. virginica* populations (Allen et al. 2003, Gaffney 2006). This strategy is predicated upon the following assumptions: (a) that disease resistance and growth are currently limiting wild population viability; (b) that extant populations are genetically ''degraded'' because of selective harvesting of larger animals; (c) that selected alleles in genetically improved oyster strains are also beneficial in nature, and (d) that these alleles can be incorporated into natural populations through strategic outplantings (Gaffney 2006).

By design, the ''genetic rehabilitation'' approach has the most severe genetic impacts on local populations on the assumption that these impacts will be positive. The whole idea, after all, is to produce oyster strains that are genetically ''superior'' and then use these strains to give natural populations an evolutionary ''boost'' towards long-term viability. Unfortunately, this strategy has significant associated risks. First among these is the possibility of incorrectly identifying traits that seem to limit population recovery, and the difficulty of developing reliable ways to measure these traits under artificial rearing conditions in the hatchery and in the field. Selectively bred strains are typically evaluated as ''cultchless singles'' grown in bags, racks, or floats, sometimes with genetic families reared separately, and it is unclear that traits like survival, growth, and fecundity measured under these conditions are correlated with the same traits under natural conditions.

Another concern is that without a thorough understanding of the sources of mortality, their interactions with environmental conditions, and the genetic potential for and constraints on their improvement, there is no guarantee that selective breeding can produce overall improvement in the phenotype. Evolutionary theory predicts that traits under strong selection in nature are expected to be mutually constrained by unfavorable genetic correlations because natural selection drives favorable genetic correlations to fixation and unfavorable genetic correlations to equilibrium (Lande 1982). If this is the case, artificial selection to improve one trait could have correlated negative effects on others (e.g., Camara et al. 2005). Conversely, if the assumption is true that a small number of specific factors such as a newly-emerged disease really does prevent population recovery, and that relevant traits are heritable and unconstrained by negative genetic correlations, then the combination of strong natural selection and ample genetic variation should result in genetic improvement without human intervention. For example, recent surveys indicate that *C. virginica* populations in some parts of the Chesapeake Bay include large individuals that may be resistant to *Perkinsus marinus*, one of the two major diseases believed to be responsible for decimating natural populations (R. Carnegie and E. Burreson, pers. comm.; http://www.growfish.com.au/content.asp?contentid=7952), raising questions about both the prudence and cost-effectiveness of hatchery-based selective breeding efforts for restoration.

In addition, selective breeding efforts typically use ''closed populations'' propagated entirely in hatcheries and genetically isolated from naturally-occurring animals, even if they are raised among them during the field ''grow-out'' phase of culture. A common feature of selectively-bred bivalve populations is that they exhibit reduced N_e, lower levels of molecular genetic diversity (allelic richness and/or heterozygosity) and higher levels of inbreeding than wild populations (Allendorf & Leary 1986, Appleyard & Ward 2006, Carlsson et al. 2006, Dillon & Manzi 1987, Gaffney et al. 1992, Gaffney & Scott 1984, Hedgecock et al. 1992, Li et al. 2006, Yu & Chu 2006, Zhang et al. 2005). As a consequence, deleterious alleles may be purged and within-population inbreeding depression reduced (Crnokrak & Barrett 2002). However, selectively-bred populations may suffer reduced fitness in the wild (i.e., among-population inbreeding depression) and/or lack sufficient quantitative genetic variation to respond to future environmental challenges. A long history of hatchery propagation provides ample opportunities for inadvertent domestication selection on larval and adult characters other than those targeted by intentional artificial selection, and it is difficult to know whether these characteristics are also adaptive in nature.

Finally, even if artificially selected strains have highly desirable characteristics such as disease resistance, their history of genetic isolation and inbreeding increases the probability that these desirable characteristics are the result of the fixation of nonadditive genetic variation (such as multigene epistatic complexes or beneficial recessive alleles). If so, restoring natural populations using these stocks will result in extensive ''hybridization'' between selected stocks and wild oysters and the inevitable breakdown of these advantages.

HOW BEST TO PROCEED?

On the one hand, there is a real need for human intervention to restore native Olympia oyster populations. On the other, the most expedient strategies pose very real risks. Fundamentally, there are two responsible approaches to dealing with these facts. The first is to wait until the data required to make a well-informed decision have been gathered. This approach is an expensive, time-consuming, and laborious process that will produce limited tangible results in the short-term and potentially frustrate the public and restoration practitioners. The second is to proceed with caution, while minimizing risks. These two approaches are not mutually exclusive. In this section, we first summarize the research required to ultimately make fully informed decisions and then outline risk-averse steps that can be taken in the near-term, even in the absence of empirical data.

Research Priorities for Informed Decision-Making

There is a great deal of debate over how to manage and restore threatened and endangered species, and reviewing this vast literature is beyond the scope of this paper. However, one

of the key concepts in this discussion is the notion of the evolutionarily significant unit (ESU), and this concept is very much relevant in evaluating restoration options. Although the concept of the ESU has changed over time (Pennock & Dimmick 1997, Waples 1998), ESUs are generally considered to be distinct populations that warrant separate management and are accorded protection under the United States Endangered Species Act. The concept can be believed as having two facets: (1) reproductive isolation and (2) adaptive distinctiveness (Crandall et al. 2000). Crandall et al. (2000) use the terms genetic exchangeability and ecological exchangeability to refer to these two facets, roughly equivalent to molecular and quantitative genetic differentiation discussed earlier. Genetically exchangeable populations are populations that are connected by ample gene flow, which is best measured using neutral molecular genetic variation. Ecologically exchangeable populations show no evidence of local adaptation, which is best evaluated by examining the level of quantitative genetic differentiation. In addition, they argue that these two aspects of population distinctiveness should ideally be understood in both recent and historical time frames. As a consequence, a fully-informed decision as to whether to manage populations as distinct and separate entities or as a single panmictic population requires four separate pieces of evidence that can be depicted as a 2×2 matrix in which ecological and genetic exchangeability form one side and recent and historical time frames form the other. Figure 1 is reproduced with permission from Crandall et al. (2000), and depicts how various combinations of genetic and ecological exchangeability in recent and historical timeframes can be used to inform management decisions. Similarly, restoration efforts must be managed to avoid disruption of extant stock structure and local adaptation.

The obvious difficulty here is that four separate lines of evidence are required to implement this in its entirety. Current data are difficult enough to obtain. However, unless the goal of restoration efforts is to recreate the past, it may be unnecessary, even inadvisable, to obtain historical data because only current ecological and genetic exchangeability contribute to future population persistence. By reducing the immediate questions to current or recent ecological and genetic exchangeability, the evolutionary significance of populations can be evaluated in terms of extant molecular and quantitative genetic differentiation. An important caveat is that simply evaluating whether populations are different at the molecular and quantitative genetic level cannot address the issue of whole-population inbreeding and/or outbreeding depression; these can only be assessed through outcrossing experiments. The research needs to make reasonable decisions on how to manage and restore native oyster populations follow.

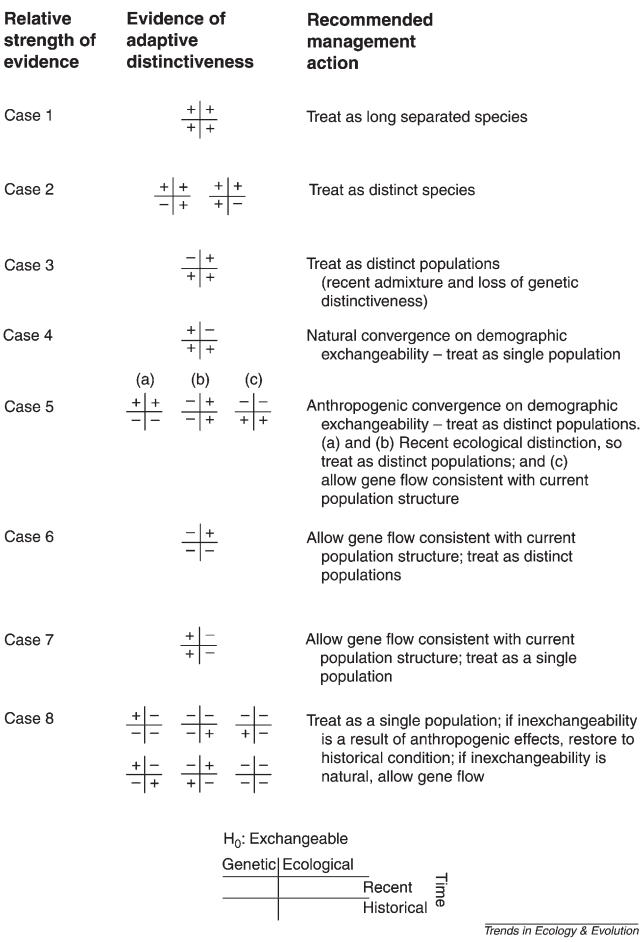

Figure 1. Categories of population distinctiveness based on rejection (+) or failure to reject (–) the null hypotheses (H_0) of genetic and ecological exchnageabilit, for both recent and historical time frames. As the case numbers increase (from Case 1 to Case 8) there is decreasing evidence for significant population differentiation.

Assessments of Molecular-level Genetic Differentiation and Gene Flow Among Extant Populations

Whereas an analysis of molecular genetic diversity within and among populations cannot be considered sufficient information to make fully informed decisions, assuming that appropriate genetic markers are available, these data are easy to obtain and can be used to address several critical questions. In particular, a molecular genetic survey can reveal the levels of gene flow between populations and their effective population sizes. Estimates of population differentiation and gene flow should ideally be obtained from temporally-spaced surveys of the populations in question (Hedgecock 1994). Likewise, effective population sizes may be best estimated from samples taken across generations (Ryman 1994, Wang 2005). A finding of little or no gene flow between populations would indicate that unless other data are counterindicative, the populations should be presumed to be (at least potentially) locally adapted and managed separately. However, because populations can be locally adapted for quantitative traits even with gene flow at neutral molecular markers, a finding of extensive gene flow cannot be used to justify managing or restoring them as a single population. As a result, because this step may be relatively quick and easy to do, it must not exclusively inform restoration decisions regarding population genetic distinctions.

Tests for Local Adaptation

There are various empirical approaches to testing for local adaptation that vary in the level of genetic detail they provide. Unsurprisingly, the amount of information produced is directly proportional to the sophistication of the experimental design and the amount of work and resources required in executing it. Another critical consideration for any test of local adaptation, however, is which phenotypic traits should be measured.

Whereas the obvious trait of interest is fitness itself, measuring fitness is extremely complicated in that it incorporates not only many facets of the postlarval phenotype, including the combination of survival, growth, and fecundity, but also larval survival and settlement success.

Absent a full assessment of fitness, measurement of individual surrogate parameters can be substituted. Taken together or individually, examples might include assessment of fecundity *via* image analysis of digitized histological sections, survivorship assessment at one or more life history stages, measurements of growth, and characterizations of temperature and/or salinity tolerance.

The quickest and most straightforward way of testing for local adaptation is to perform reciprocal transplant experiments. These experiments can be quite simple in design if they focus only on population-level effects and do not incorporate crosses between populations or among-family effects within populations to simultaneously estimate within population genetic variation and test for inbreeding and outbreeding depression (see later), Unfortunately, these experiments run the risk of resulting in gene flow among populations at the same time as they determine whether such gene flow would be problematic.

One approach to avoiding transplantation into existing populations would be to estimate Q_{ST} and F_{ST} using a common garden approach (described later) at a site with suitable habitat but no native population. However, if, for some reason, this is impossible or impractical, molecular markers can be used to estimate Q_{ST} *in situ*. This involves using the multilocus genotypes gathered as part of a molecular genetic survey to reconstruct the genetic relationships among individuals within samples (see earlier) and then using this family structure to estimate Q_{ST}. Whereas the major advantage of this approach is that it eliminates the need for hatchery production of pedigreed families and for transplantation of these families into either common gardens or existing populations, there are also several severe drawbacks. As discussed earlier, estimates of the genetic variation measured in two populations experiencing two different environments are potentially confounded with population-specific environmental components of variation that cannot be estimated or partitioned. As well, there is no guarantee that without an experimental breeding design, collections from wild populations will include enough relatives to provide reliable estimates of Q_{ST}.

If a suitable site can be identified, a common garden can be used to estimate Q_{ST}. The biggest advantages of the common garden approach are (1) simplicity: only one environment is necessary, and (2) avoidance of translocating animals among potentially locally adapted populations if the experiment is conducted with adequate safeguards. These safeguards include: (1) to conduct the experiment at a site with no natural populations, (2) to conduct the experiment under quarantine conditions (e.g., trays or troughs that receive raw seawater inputs but all effluent is treated to prevent the escape of gametes), (3) to limit the duration of the experiment to prereproductive life stages, and (4) to use sterile animals such as genetic triploids. Unfortunately, the quarantine and sterilization approaches have their own limitations. Conducting experiments in trays or troughs necessarily eliminates many potentially critical ecological factors such as competitors and predators. Sterile animals, by definition have no fitness, and at least potentially reallocate resources normally used for reproduction to other functions with unknown impacts on other components of fitness.

Assuming that suitable common garden conditions can be achieved, a minimal experimental design would consist of arrays of nested full- and half-sib families from each of the populations under study. Full-sib arrays are simpler to execute, but cannot separate additive from nonadditive genetic variance. In either case, however, it is absolutely necessary to be able to determine the parentage of individuals. This can be accomplished by either rearing families separately through the entire experiment or by mixing families for rearing, and reconstructing pedigree information either by tagging individuals or through genetic parentage assignment using molecular markers.

Evaluating the levels of quantitative genetic variation within and among populations in a single, common environment eliminates some, but not all of the difficulties caused by the confounding of genetic and environmental variation. There are still potential problems with genotype-by-environment interactions that could influence the estimates of quantitative genetic variance, but these can only be truly be evaluated by reciprocal transplant experiments that also incorporate a mating design (see later).

If neutral molecular markers tightly linked to QTL for fitness traits or, even better, polymorphisms in the specific genes that control important ecological traits can be identified, then comparing patterns of variation within and among populations at these QTL to the patterns at neutral loci should provide information about local adaptation. The *Lap* locus in mussels (see earlier) provides a single-locus example. More powerful approaches, however, involve genome-wide approaches. One intriguing possibility, is the idea of "hitchhiking mapping" to identify neutral genetic markers that are closely linked to QTL or candidate genes and contribute to population differentiation (Schlötterer 2002, Schlötterer 2003). This approach requires a large number of polymorphic markers that can be assembled into a linkage map, not a trivial undertaking, but something that is very achievable in various marine invertebrates (e.g., Hedgecock et al. 2003, Hubert & Hedgecock 2004, Li & Guo 2004, Liu et al. 2005, Yu & Guo 2003, Zhou et al. 2006). Recently, however, Bonin et al. (2007) suggested a potentially powerful and alternative approach that uses logistic regression to test for associations between amplified fragment length polymorphisms (AFLP) and environmental factors. Because AFLP markers require no prior sequence information and very little development compared with other types of molecular markers, this approach may be both cost effective and practical for nonmodel organisms such as Olympia oysters.

Another level of analysis is to directly compare the DNA sequences of genes involved in ecologically important traits among populations with the aim of identifying the functional differences among alleles. The biggest problem with this approach is that it requires a great deal of prior knowledge of the genome. Whereas this level of sequence information could be available soon for commercially important oyster species (Hedgecock et al. 2005), and a DNA microarray of both Pacific and eastern oyster genes has recently been developed (Jenny et al. 2007) it will be quite some time before this approach is feasible in other oyster species, including Olympia oysters.

The experiments outlined above are adequate to address whether populations are genetically differentiated with respect to both neutral molecular genetic variation and adaptive quantitative genetic variation. However, they cannot determine whether distinct populations suffer from among-population inbreeding depression which could be alleviated by genetically mixing or whether this genetic mixing would disrupt local adaptation through outbreeding depression. These issues can be readily incorporated into common garden experiments, but require a substantial increase in hatchery work. In addition to the within-population crosses above, among-population matings must be incorporated into the experimental design. *A priori* linear contrasts comparing within-population to among-population crosses would thus provide direct tests for positive and negative effects of crossing among populations (e.g., Camara et al. 2006).

All of the local adaptation experiments described earlier assume that the results obtained in a common garden can be extended to the full range of a species' natural habitats. Technically, this amounts to assuming that there are no consistent genotype-by-environment interactions such that the performance rankings of populations change from environment to environment. Unfortunately, this is entirely an empirical question. The best test of this assumption would be to plant family arrays from all of the populations under study into all of the environments under study, a truly massive undertaking for anything over two or three populations. One way to reduce this workload would be to identify *a priori*, important environmental gradients and to either recreate these conditions in the laboratory or choose a small subset of field sites that best represent these gradients. As with test of local adaptation earlier, the choice of traits to measure is critical.

Measures that can be Cautiously Implemented Immediately

Clearly, the "Do Nothing" and "Habitat Restoration" options have almost no genetic impacts, and it is difficult to imagine how implementing these strategies could cause genetic-level problems. Assuming that Olympia oyster populations were historically large and on at least small spatial scales highly connected by gene flow, restoring habitat is unlikely to produce inappropriate gene flow, the only real cause for concern. In contrast, supplementing existing populations or establishing new populations can have much larger genetic impacts and should be implemented only cautiously in the absence of empirical data. As discussed earlier, population supplementation efforts can produce negative genetic consequences in two basic ways: (1) among-population effects from inappropriate gene flow between locally adapted populations and (2) within-population effects caused by reductions in effective population size and genetic diversity, and through inbreeding depression.

Minimizing Among-population Effects

Among-population effects can only be addressed empirically through studies of local adaptation and outbreeding depression. In the absence of reliable data, a precautionary approach would be to assume that populations are locally adapted and genetically isolated, to manage and restore them separately until data become available, and even then only if the populations show no evidence of local adaptation and outbreeding depression. The consequences of mixing populations inappropriately cannot be easily reversed, whereas mixing is easily accomplished at any time should the data indicate that this is appropriate. Thus, transplantation and hatchery supplementation of existing populations should rely upon locally-derived broodstock whenever possible unless evidence indicates that mixing populations is advisable. The seeding of new populations should use broodstock from either the closest source population or a population that experiences very similar ecological conditions, preferably both.

Minimizing Within-population Effects

In terms of the within-population effects, the potential for harm is mainly related to reduced N_e and inbreeding depression and/or inadvertent domestication selection during hatchery propagation. Two primary management strategy categories can be implemented to increase genetic diversity in cultured outplants and circumvent any negative genetic effects of hatchery culture. These strategies focus on (1) maximizing the effective population size or more specifically the ratio N_e/N and (2) maximizing genetic diversity. A third strategy would be to create a culture environment as similar as possible to the natural environment. This strategy will not be addressed here, because eliminating differences between the hatchery and natural environments is difficult at best (Busack & Currens 1995) and inconceivable for marine molluscs such as the Olympia oyster. If, however, bivalves are exposed to the hatchery environment for only a single generation, domestication selection during the relatively short hatchery period is likely to be of much less concern than overall loss of genetic variability (Utter 2004).

Maximize N_e/N

Ryman et al.(1995) suggest that the variance effective population size is the most important parameter to concentrate on when genetic interactions between cultured and wild are expected because this dynamic is associated with the loss of genetic diversity. Thus, maximizing the N_e/N ratio in the hatchery can maximize the genetic variability in the cultured stock. Several practical measures can help in this regard:

Maintaining a 1:1 Sex Ratio

Significant deviations from a 1:1 sex ratio of reproductively successful parents have a profoundly negative effect on effective population size because the less abundant sex represents, in essence a genetic bottleneck since half of the population's genes must be transmitted through each sex (Conner & Hartl 2004). Distorted sex ratios may occur because of unequal sex ratios in the population or as a consequence of the mating system. Wild adults may be found in roughly equal sex ratios, but the mass spawning mating system undoubtedly distorts the sex ratio of effective parents and thus the N_e/N ratio. Hatchery mating systems should be designed to minimize deviations from a 1:1 effective sex ratio, which will maximize hatchery N_e. To equalize the sex ratio, discrete pair matings should be conducted in pairwise, or factorial crosses.

Reducing Family Size Variance

It is insufficient to merely conduct single pair matings. Outplanting equal numbers of progeny from each cross maximizes effective population size. Differential family survival reduces effective population size, so to maintain maximum

effective population size in the hatchery, the variance in family size must be kept to a minimum. To ensure equal contribution from each cross (family), families should be maintained separately until outplanted. At a minimum, if different family groups are raised in a common garden, before outplanting, a large sample of the seed should be genotyped (as for the broodstock, earlier) to ascertain the distribution of family sizes and estimate the effective population size. If no particular family or set of families dominates the population, the effects of any genetic interaction will be minimized. In nature, the ratio of effective to census population size is, on average, 0.11 (Frankham 1995b). Also, to the extent family size variance can be kept at a minimum, selection can be reduced (Allendorf 1993).

Maximize Genetic Diversity

Maintaining adequate levels of molecular and quantitative genetic variation is essential to avoid inbreeding and inbreeding depression as well as ensuring that populations have the ability for adaptive responses to changing conditions. The following measures are recommended:

Procure New Wild Broodstock Frequently

Instead of developing closed broodstock lines, which would result almost certainly in genetic change over time, using a different set of wild broodstock for each spawning can help maximize representation of the wild gene pool. This management strategy runs contrary to typical molluscan culture operations, especially those used for selective breeding. By avoiding repeated spawns of the same broodstock and by obtaining new wild broodstock for each successive spawn, the genetic diversity present in wild populations will be better reflected in seedstocks (given adherence to other recommended practices).

Minimize Inbreeding

If natural populations show high levels of heterozygosity and genetic diversity, and if hatchery procedures are implemented to maximize the N_e of outplanted seed, then procuring new broodstock frequently should also result in very low levels of inbreeding. However, if extant populations are small and/or if new broodstock collections may include the progeny of previous outplantings, there is the potential for substantial levels of inbreeding. In such cases, broodstock should be genotyped prior to spawning so that parents with rare genotypes can be prioritized for inclusion and matings can be conducted between pairs of maximally unrelated individuals. Identifying parents with rare genotypes could be straightforwardly accomplished by estimating the average pair wise relatedness between each potential parent and all other potential parents and giving priority to individuals with the lowest overall relatedness or ''mean kinship'' to the rest of the population (Ballou & Lacy 1995, Doyle et al. 2001, Sekino et al. 2004). Minimizing inbreeding once a set of parents has been identified is a simple matter of estimating the pairwise relatedness among all potential parents and giving priority to pairings that minimize parental relatedness. Pairwise relatedness estimates are notoriously imprecise (Blouin 2003), but precision can be increased either by increasing the number of loci, or number of alleles per locus. For these reasons and to minimize the cost of genotyping, highly variable, codominant loci such as microsatellites would be preferable to either less variable markers (e.g., SNPs) or dominant markers (e.g., RAPDs or AFLPs). This, however, must also be balanced against the problems caused by null alles (Hare et al. 1996, Hedgecock et al. 2004).

SUMMARY AND CONCLUSIONS

Restoration of native Olympia oysters and other bivalves requires careful consideration of the potential for beneficial and harmful genetic impacts. The most important considerations from a genetic perspective are the levels of adaptive genetic variation harbored by existing remnant populations, its distribution among populations, the degree to which populations are locally adapted, and the genetic mechanisms that produce local (and global) adaptation. These factors contribute to the adaptive potential of populations and thus their long term persistence and also determine the consequences of interbreeding between distinct populations. All are best investigated through studies of quantitative genetic variation. Of less direct utility is an understanding of the levels of molecular genetic variation within and among populations. Whereas a molecular genetic approach can tell us whether populations are currently reproductively isolated and/or differentiated, differentiation at this level could be the result of either historical separation and local adaptation or recent anthropogenic fragmentation leading to low N_e, random genetic drift, and inbreeding. Unfortunately, these different historical and mechanistic paths to population differentiation at molecular markers have completely opposite implications for management and restoration.

As a consequence, an increasing number of researchers are questioning the use of molecular genetic data for conservation and management. Whereas there are very few studies that address local adaptation in bivalves, what little research is available indicates that there is considerable potential for local adaptation, (e.g., Luttikhuizen et al. 2003) even with high levels of gene flow (Koehn et al. 1980, Koehn & Hilbish 1987 and references therein), and studies in other organisms indicate that studying only molecular level patterns can be misleading (e.g., Bekessy et al. 2003).

Restoration options focused on restoring habitat and relying on natural recruitment have the least potential for unintended and potentially harmful genetic impacts. Whereas this could produce inappropriate gene flow between reproductively isolated, locally adapted populations with incompatible genetic compositions, this seems unlikely. Restoration options that use population supplementation, whether through hatchery production or the translocation of natural spatfall, carry higher risks and should be carefully studied before implementation.

Because of the relative ease of data collection, genetic studies typically focus first on describing molecular genetic variation within and among populations. If analyses of selectively neutral molecular genetic variation indicate that populations are, at present, genetically differentiated and reproductively isolated, a precautionary approach is to assume that populations are also locally adapted and that restoration efforts that create unnatural gene flow between populations may disrupt these adaptations through outbreeding depression. If, on the other hand, molecular genetic data indicate that populations are connected by extensive gene flow at neutral loci, then transplantations among populations are unlikely to do much harm. Unfortunately,

it cannot be simply assumed that they will do much good either. If strong local selection and adaptation quickly eliminate the imported genotypes, they will contribute little to subsequent recruitment, wasting a great deal of effort that could be better directed toward more effective measures.

If hatcheries are used for restoration purposes, careful attention to managing their impacts on neutral and adaptive genetic diversity is paramount. At the molecular level, this attention can range from relatively simple practices such as selecting appropriate parents and making concerted efforts to equalize their contribution to the population to sophisticated monitoring of inbreeding and relatedness using molecular markers. At the quantitative level, at a minimum, the performance of hatchery-reared juveniles should be monitored and compared with wild populations. If hatchery-produced juveniles represent nonlocal stocks or have been subjected to intentional selection for desirable characteristics, sophisticated crossing experiments to evaluate the consequences of these practices are required for sound decision-making.

Unfortunately, this is no simple task. The impacts of hatchery-based restoration efforts on adaptive genetic variation are difficult to study and all too easy to ignore. Allen et al. (2003), for example, promote a strategy that they call ''terra-forming'' through ''genetic rehabilitation'' of native oyster populations in the Chesapeake Bay at a truly massive scale, even while admitting that we have a ''limited understanding of the overall dynamics of genetic rehabilitation or even its prognosis for success.'' That prognosis for success, we believe, should be evaluated using quantitative genetic approaches, preferably before committing large sums of funding to such costly and high-profile efforts. Unfortunately, the relative ease of acquiring molecular genetic data and the dearth of funding for low-tech research and the hatchery infrastructure to support it pose serious challenges. Proceeding in ignorance may well be the only option available unless the scientific community makes filling the present gaps in our knowledge a priority.

At present, this does not seem to be occurring. For example, two recent reviews of the genetic risks associated with hatchery supplementation of finfish and shellfish populations (Gaffney 2006, Hedgecock & Coykendall 2007) heavily emphasize effective population size (N_e) and address adaptive, quantitative genetic variation only briefly. Gaffney (2006), similar to Allen et al. (2003) mentions only in passing that little is known about the genetic basis of adaptation in oysters *en route* to advocating the use of stocks selected for enhanced disease resistance under aquaculture conditions to supplement native oyster populations in the Chesapeake Bay, but goes even further by proposing a novel but untested strategy using F_1 hybrid progeny between cultured and wild stocks. Several critical but as yet untested assumptions regarding the genetic basis of the presumed desirable phenotypes of cultured strains must, however, hold true in order for this strategy to be effective: (1) The metrics used to measure the traits targeted for improvement in closed hatchery populations in which larvae have been reared exclusively in hatcheries for multiple generations and juveniles and adults have been grown as ''singles'' with no attachment to hard substrate in protective bags or cages to exclude predators with periodic rotation and cleaning of fouling organisms are reliable indicators of performance under natural field conditions; (2) Selective breeding for targeted traits has not resulted in undesirable correlated responses in other ecologically important characters; (3) F_1 and more advanced hybrid progeny between hatchery and wild stocks will show hybrid vigor rather than outbreeding depression; (4) The functional genes (QTL) for traits improved through artificial selection can easily and effectively be introgressed into wild populations. Although future research may demonstrate that all of these assumptions are correct, at present, there is no evidence to justify them and thus the implementation of this novel strategy.

The Olympia oyster is currently not in danger of extinction and at this time is not an economically important species. Research into how best to restore the species and ''on-the-ground'' restoration efforts are at a very preliminary stage. As a consequence, the situation on the west coast of the United States is much less desperate than on the east coast, especially Chesapeake Bay, and there is much less pressure to demonstrate immediate and dramatic results to dissipate public and political frustration with several decades of publicly-funded research that has failed to noticeably increase oyster populations (Mann & Powell 2007). West coast oyster restoration efforts have the luxury of time and of learning from past mistakes, and an opportunity to ''get it right'' by rigorously studying extant populations and evaluating alternative strategies before they are implemented.

At present, we know almost nothing about how molecular or quantitative genetic variation is distributed among Olympia oyster populations or how past human activities have affected these patterns. Microsatellite markers suitable for this species have only recently been developed (Stick et al. 2009), and hierarchical analyses of neutral genetic variation within and among west coast estuaries using these markers are currently in progress (Stick et al. 2008). However, a great deal more research is required before we can proceed with confidence. To make the most impact with the limited resources available for Olympia oyster restoration, it is imperative that we restrict restoration activities to those that have the lowest risks of unintended negative consequences at the genetic level until we develop a better understanding of how natural populations currently function and the impacts of more aggressive approaches to restoration. The difficulty is balancing the best of intentions against the risks of making things not better, but worse.

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